Article

Pathogenesis of primary hypercalciuria

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Summary

Hypercalciuria may be classified into absorptive, renal and resorptive forms, depending on whether the primary defect is intestinal hyperabsorption of calcium, renal leak of calcium, or excessive bone resorption. In absorptive hypercalciuria, the pathogenetic role of vitamin D is uncertain, and mutations in the chloride channel may occur mainly in association with Dent's disease. Early studies suggest that a new soluble adenylyl cyclase (*AHRAC*) may be etiologically important in this condition, since base changes in this gene occur much more frequently and are directly correlated with intestinal calcium absorption.
The distal nephron is the site of reabsorption of the final 20% of filtered calcium. The transcellular reabsorption of calcium begins with a passive entry of Ca^{2+} through apical calcium channels, followed by diffusion through cytosol and active extrusion across the basolateral membrane. Calcium transport in the nephron is regulated by luminal pH, calcitriol, estrogen, parathyroid hormone, prostaglandin E_2, and sodium load. A biochemical picture of renal hypercalciuria may be produced by acid load from dietary animal proteins, prostaglandin E_2 excess, sodium load, hypoparathyroidism, and estrogen deficiency. So far, mutations in apical calcium channel have not been found.
The hallmark of resorptive hypercalciuria is primary hyperparathyroidism. Bone loss often accompanies absorptive hypercalciuria. *AHRAC* may be implicated, since base changes in this gene are inversely correlated with spinal bone density. Dietary acid load from high animal protein diet may cause hypercalciuria, in part by stimulating bone loss.

KEY WORDS: primary hypercalciuria, renal calcium excretion, intestinal calcium absorption.

Introduction

Hypercalciuria is clinically important since it often accompanies the formation of calcium-containing kidney stones. Among patients with idiopathic calcium oxalate nephrolithiasis, hypercalciuria is the main determinant for the formation of calcium phosphate nidus (in the thin loops of Henle of the nephron) that may initiate calcium oxalate crystallization (1). The correction of hypercalciuria by thiazide or indapamide has been reported to reduce the rate of recurrent stone formation (2). In a risk analysis, hypercalciuria confers a higher risk for stone formation than hyperoxaluria (3).
The pathophysiological basis for hypercalciuria is multifactorial, involving disturbances in calcium handling at three organs - intestine, kidneys and bone. Accordingly, hypercalciuria has been classified into absorptive, renal and resorptive forms, depending on whether the principal defect is intestinal hyperabsorption of calcium, "renal leak" of calcium or excessive bone resorption (4). This article will review recent advances in the pathophysiology of each of three main causes of hypercalciuria. It is understood, however, that a primary defect in calcium handling in one organ system may produce a secondary disturbance in other organ system. Moreover, in some conditions, calcium handling may be primarily disturbed in more than one organ systems.

Pathophysiology of absorptive hypercalciuria (Table I)

Table I - Pathophysiology of Absorptive Hypercalciuria.

1. General Description
2. Potential Pathogenetic Role of Vitamin D
3. Genetic Basis of Absorptive Hypercalciuria
   a. AHRAC Gene
   b. Chloride Transporter
4. Sarcoidosis

General Description

Absorptive hypercalciuria (AH) describes a stone-forming condition in which the primary defect is presumed to be enhanced intestinal absorption of calcium (4,5). The increased absorbed calcium transiently raises serum calcium and suppresses parathyroid function. Hypercalciuria ensues from the increased renal filtered load of calcium, and decreased renal tubular reabsorption of calcium due to parathyroid suppression. In the classic presentation (AH Type I), the syndrome is characterized biochemically by normocalcemia, normal or low serum parathyroid hormone (PTH), high intestinal calcium absorption, and hypercalciuria. Urinary calcium is high (>200 mg/day) on a diet restricted in calcium (400 mg/day) and sodium (100 mEq/day), and remains high (>300 mg/day) on a high calcium

diet. In normal subjects, it is <200 mg/day on a low calcium diet and rarely exceeds 250 mg/day on a high calcium diet (4). Fasting urinary calcium is normal, and is appropriate for the level of parathyroid function. The intestinal hyperabsorption of calcium is unaffected by reduction of urinary calcium by thiazide, or alteration of 1,25-dihydroxyvitamin D [$1,25(OH)_2D_3$] synthesis or sensitivity by orthophosphate or steroid (5).

AH may present itself in a less severe form (AH Type II), wherein urinary calcium is normal on a calcium-restricted diet, though elevated on a high calcium diet. It may also occur in a severe form (fasting hypercalciuria), in which fasting urinary calcium is high. Fasting hypercalciuria may reflect inadequate duration of fast, incomplete renal clearance of absorbed calcium or reduced renal tubular reabsorption of calcium from suppressed parathyroid function. In some patients, however, fasting hypercalciuria may be reflective of concomitant bone loss. Although radial shaft bone density is spared, spinal bone density has been reported to be reduced in AH type I and fasting hypercalciuria (6).

Potential Pathogenetic Role of Vitamin D

The pathogenetic role of vitamin D in AH is uncertain. Serum $1,25(OH)_2D_3$ concentration has been reported to be either high or inappropriately high. A biochemical-physiological picture of AH can be produced by administration of $1,25(OH)_2D_3$ to normal subjects. In some patients with AH, an inhibition of $1,25(OH)_2D_3$ synthesis by a short-term treatment with ketoconazole reduced intestinal calcium absorption and urinary calcium (7). However, calcium absorption was shown to be increased in the jejunum but not in the ileum or colon among patients with AH, whereas it was stimulated in all intestinal segments following treatment with $1,25(OH)_2D_3$ (5).

In the genetic hypercalciuric rats stone-forming, $1,25(OH)_2D_3$ receptors (VDR) in the intestine were two-fold higher than in normocalciuric control rats (8), but VDR mRNA was not increased (9). This increase in VDR was associated with a higher amount of calbindin-D_{9k}, occurring primarily or secondary to high VDR. Thus, in this animal model of AH, hypercalciuria may be due to vitamin D-dependent stimulation of intestinal calcium absorption.

In human beings, no increase in VDR number or affinity for the ligand for the receptor was found in monocytes and activated T cells or in cultured skin fibroblasts from AH patients (10). There was neither detectable mutations in the VDR coding sequence nor an association of VDR polymorphisms with increased intestinal calcium absorption (11). Finally, linkage analysis failed to implicate the VDR locus on chromosome 12 with the AH phenotype (12). However, in another study, quantitative trait analysis of urinary calcium excretion revealed linkage to some but not all markers in the VDR region (13). The same study eliminated the 1β-hydroxylase gene as being associated with hypercalciuria.

Genetic Studies in Human Beings with AH

AHRAC Gene. Prior studies have indicated that the inheritance of AH is compatible with an autosomal dominant trait. Reed et al. identified a locus on chromosome 1q23.3-24 in three kindreds with phenotypically well-defined AH (12). Within this region, they identified a candidate gene, the absorptive hypercalciuria-related adenylyl cyclase (*AHRAC*), on account of its association with AH (14) and the cyclase function in the soluble cytosolic cellular fraction described in its rat ortholog (15). The gene is ubiquitously expressed in humans and a number of base changes have been identified. While some of these base substitutions in *AHRAC* can be found in the normal population, the frequency in base changes was higher in patients with AH (14). Figure 1 summarizes the allelic frequency of all the base changes combined. Although one can easily find single base changes in normal subjects, most patients with >4 base changes have clinical AH.

Past studies have shown that *AHRAC* clearly encodes an adenylyl cyclase and is expressed in the intestine. However, it is not well understood at present how *AHRAC* regulates intestinal calcium transport and how the various polymorphic variants lead to hyperabsorption of calcium. At the empirical level, the intestinal absorption is positively correlated with the number of base changes in *AHRAC* (Fig. 2). This finding strongly suggests that *AHRAC* may control intestinal calcium absorption. It is important to state that intestinal calcium absorption is a continuous variable under polygenic as well as non-genetic control. The elucidation of the function of wild type and variant AHRAC in the gut should advance our knowledge of the pathophysiology of AH.

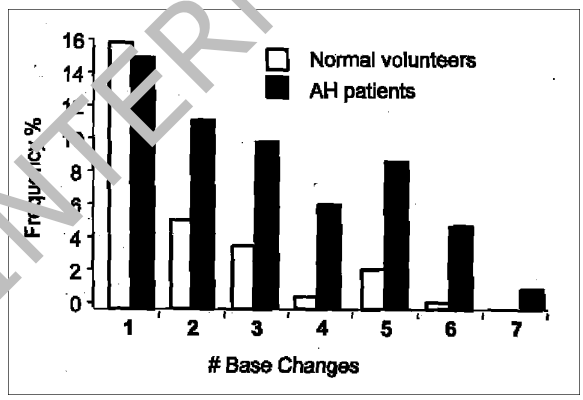

Figure 1 - Percentage of individuals harboring from 1-7 base changes in *AHRAC*. Open bars depict normal volunteers (n = 155) and closed bars indicate AH patients (n = 135).

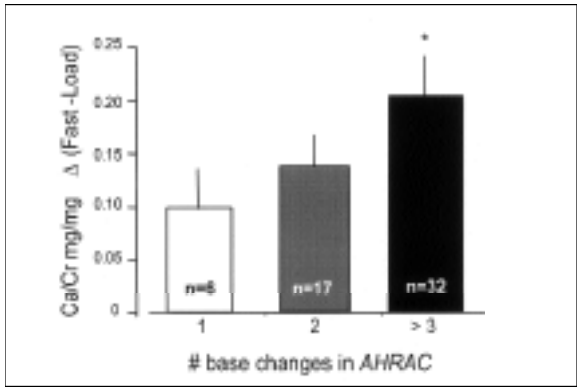

Figure 2 - The relationship between calciuric response to oral calcium load and number of base changes in *AHRAC* Phenotype. A 2-h fasting urine collection was obtained for measurement of calcium and creatinine. After a synthetic meal containing 1 gm calcium, a 4-h urine collection was obtained for calcium and creatinine. The difference (Δ) between urinary calcium post-calcium load and fasting urinary calcium is a surrogate of intestinal calcium absorption. N = number of subjects studied. Asterisk denotes statistically significant difference from subjects with 1 base change ($p<0.05$ by ANOVA).

Chloride channel. Dent's disease (X-linked nephrolithiasis) is a condition with hypercalciuria of yet unresolved pathogenesis and "low molecular weight proteinuria" (urinary loss of proteins of low molecular weight). Dent's disease is caused by mutations in the CLCN5 chloride channel. Although low molecular weight proteinuria is highly prevalent in this disease, one individual with documented CLCN5 mutation was found to have hypercalciuria without proteinuria, raising the possibility that CLCN5 mutations may underlie some patients with calcium nephrolithiasis (16). Scheinman et al. found some degree of low molecular weight proteinuria in 9% of patients with hypercalciuria but failed to show base changes in CLCN5 from genotying 32 patients (16). Analysis of the genetic hypercalciuric rats with stones also failed to show base changes in CLCN5. If CLCN5 defects cause calcium nephrolithiasis outside the context of Dent's disease, the incidence is likely very low.

Sarcoidosis

Sarcoidosis is a granuloma-forming disorder characterized by mild to severe hypercalcemia in 10% of patients, with hypercalciuria occurring in up to 50% of patients at some time during the course of their disease. For many years, it was believed that hypercalcemia and/or hypercalciuria resulted from increased sensitivity to the biological effects of vitamin D. Subsequently, the circulating concentrations of $1,25(OH)_2D_3$ was found to be high, due to its extrarenal synthesis by macrophages in sarcoid granulomata (17). Thus, the increased $1,25(OH)_2D_3$ synthesis causes hypercalcemia and hypercalciuria by stimulating intestinal calcium absorption and stimulating bone resorption.
The distinction between AH and sarcoidosis is best exemplified by the glucocorticoid response. Glucocorticoids are effective in the management of hypercalcemia of vitamin D toxicity and hypercalciuria associated with sarcoidosis and other granulomatous conditions. Among patients with sarcoidosis, prednisolone treatment significantly decreased serum $1,25(OH)_2D_3$ and intestinal calcium absorption, whereas this treatment was uniformly ineffective among patients with AH (18).

Pathophysiology of renal hypercalciuria (Table II)

Table II - Pathophysiology of Renal Hypercalciuria.

1. General Description
2. Physiological and Molecular Basis of Renal Hypercalciuria
   a. Calcium Transport in the Nephron
   b. Apical Calcium Channels
3. Regulators of Calcium Transport in the Nephron
   a. Luminal pH
   b. $1,25(OH)_2D_3$ and Estrogen
   c. Prostaglandin E_2
   d. Sodium Load
4. Clinical Conditions Associated with Renal Hypercalciuria
   a. High Animal Protein Diet
   b. Dietary Acid Load
   c. PGE_2 Excess
   d. Dietary Sodium Load
   e. Relative Hypoparathyroidism from Enhanced Intestinal Ca Absorption
   f. Estrogen Deficiency of Postmenopausal State
   g. Mutations of ECaC Genes as a Potential Cause of Renal Hypercalciuria

General Description

Renal hypercalciuria is an uncommon cause of hypercalciuric nephrolithiasis. It is believed to result from a primary impairment in the renal tubular reabsorption of calcium (5). The resulting transient decline in serum calcium concentration stimulates parathyroid function, which in turn enhances $1,25(OH)_2D_3$ synthesis and intestinal calcium absorption. Biochemically, serum calcium is normal and fasting urinary calcium is high, coincident with elevated serum PTH, indicative of secondary hyperparathyroidism from renal calcium leak. A disturbed function of renal proximal tubule was suggested by exaggerated calciuric response to carbohydrate ingestion, and accentuated natriuresis to thiazide challenge (19). Unlike in AH, the correction of renal calcium leak by thiazide restores normal parathyroid function and intestinal calcium absorption (5).

Physiological and Molecular Basis of Renal Calcium Transport

Calcium Transport in the Nephron. The kidney is critical for maintaining calcium homeostasis. To maintain calcium balance, about 98% of calcium load filtered by the glomerulus must be reabsorbed along the nephron. Approximately 70-80% of the filtered load of calcium is reabsorbed in the proximal tubule and the thick ascending limb (TAL) of Henle's loop. The reabsorption of calcium in the proximal tubule and TAL occur passively through the paracellular pathway. The remaining about 20% of calcium reabsorption in kidney occurs via a transcellular pathway in the distal part of the nephron, consisting of distal convoluted tubules (DCT), connecting tubules and the initial portion of the cortical collecting ducts.
The transcellular reabsorption of calcium in the distal nephron is a multi-step process (Fig. 3). It begins with passive entry of Ca^{2+} through Ca^{2+} channels in the apical membranes, followed by diffusion of Ca^{2+} through cytosol facilitated by binding to the $1,25(OH)_2D_3$-dependent Ca^{2+}-binding protein (calbindin-D_{28K}), and eventually by extrusion of Ca^{2+} across the opposing basolateral membranes. The extrusion of Ca^{2+} across the basolateral membranes requires energy and is mediated by $Na+/Ca^{2+}$ exchangers and Ca^{2+}-ATPases operating against the electrochemical gradient for Ca^{2+}. It has been postulated that the ini-

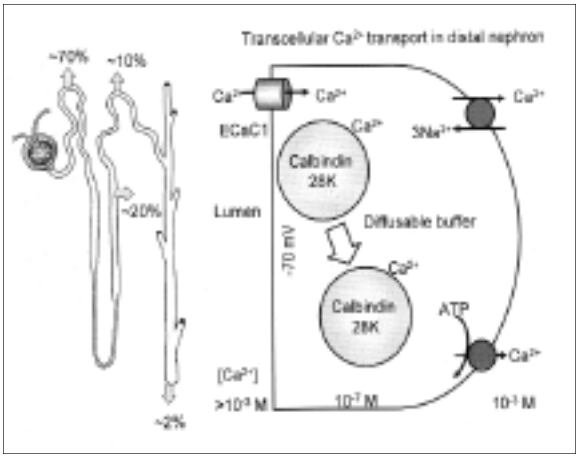

Figure 3 - Renal calcium handling. Left panel: Relative absorptive rates are shown as percentage of ultrafilterable calcium at the glomerulus. Right panel: The scheme for transcellular transport of calcium in the distal renal tubule. The apical entry step via ECaC1 is directly gated by

tial passive entry through Ca^{2+} channels in the apical membranes is likely the rate-limiting step of the trans-epithelial reabsorption of calcium in the distal nephron (20).

Apical Calcium Channels. The cDNAs for the apical Ca^{2+} channels in the distal nephron have been recently isolated. These are called ECaC1 (for epithelial Ca^{2+} channel-1) and ECaC2 (also known as TRPV5 and TRPV6 respectively). In the kidney, TRPV5 and TRPV6 are localized to the apical membranes of distal nephron (21).

Regulators of Calcium Transport in the Nephron

Although the active transcellular reabsorption of calcium in the distal nephron accounts for only about 20% of total reabsorbed calcium, it is the major target for regulation by key factors implicated in the development of hypercalciuria.

Luminal pH. Using patch-clamp electrophysiological recording of recombinant EcaC1 channels expressed in cultured cells, low extracellular pH directly inhibited ECaC1 channel activity with an apparent pK_a of 6.55 which is within the physiologic range of luminal pH of the distal nephron (22). The direct inhibition occurred as a result of extracellular proton titration of glutamate-522 (rabbit ECaC1) in an extracellular loop. The ECaC1 activity was inhibited by 16% for a drop in extracellular pH of 0.4.

$1,25(OH)_2D_3$ and Estrogen. $1,25(OH)_2D_3$ stimulates calcium reabsorption via genomic mechanisms analogous to classical steroid hormones. It increases the mRNA levels of both calbindin-D_{28K} and the Na^+/Ca^{2+} exchanger in a time frame that requires a genomic mechanism (23). While not expressed as high a level as calbindin-D_{28K}, calbindin-D_{9K} is also expressed in the kidney and upregulated at the mRNA level by $1,25(OH)_2D_3$. Recently, $1,25(OH)_2D_3$ was shown to regulate the expression of ECaC2 in the kidney (24). $1,25(OH)_2D_3$ may also influence calcium reabsorption by altering the expression levels of the PTH receptor and 25-hydroxyvitamin D-24-hydroxylase (23).
Until recently estradiol has not been considered a calcitropic hormone. However, recent studies in animal models of estrogen deficiency indicate that estrogens might have an effect on calcium reabsorption in the kidney and calcium absorption in the intestine independently of vitamin D. One model utilized aromatase knockout (ArKO) mice. Aromatase synthesizes estrogens from androgen precursors. ArKO mice are deficient in estrogens but not other gonadal factors. The ArKO female mice had elevated urinary calcium/creatinine ratios despite high serum PTH indicative of renal calcium leak (unpublished observations, Preisig et al.). Compared with the wild type mice, the female ArKO mice had significantly lower expression of calbindin-D_{28K}, ECaC1, ECaC2, Na^+/Ca^{2+} exchanger and plasma membrane Ca^{2+} pump, and lower protein levels of calbindin-D28K, Na^+/Ca^{2+} exchanger, and plasma membrane Ca^{2+} pump in the kidneys (25,26). These data strongly support the possibility that renal calcium leak is present in estrogen deficiency through alteration in the expression or synthesis of molecules involved in calcium transport in the distal nephron.
The above role of estrogen is supported by other studies in experimental animals. Estradiol therapy in ovariectomized rats increased the renal expression of calbindin-D_{28k} without changing serum $1,25(OH)_2D_3$ and ECaC1 mRNA (27). Moreover, estradiol treatment of mice deficient in the 1α-hydroxylase or VDR significantly increased ECaC1 mRNA, indicating a $1,25(OH)_2D_3$-independent stimulatory effect of estradiol.

Parathyroid Hormone. The mechanism by which PTH augments renal tubular reabsorption of calcium is not completely understood. PTH is a potent inhibitor of proximal bicarbonate reabsorption but causes minimal or modest bicarbonaturia (28,29). Direct effect of PTH on the distal convoluted tubule has been postulated but exactly how PTH increases calcium transport in this segment is unknown (30). It is attractive to speculate that the rise in luminal pH, due to the shift of bicarbonate reabsorption from the proximal to the distal nephron, stimulates ECaC activity (22) and enhances reabsorption of calcium.

Prostaglandin E_2 (PGE_2). PGE_2 has been shown to inhibit Ca^{2+} transport in DCT (23). While the exact mechanism is unknown, type-3 prostaglandin E_2 (EP_3) receptor may activate phospholipase C, which may then decrease phosphatidylinositol 4,5-bisphosphate (PIP_2) concentration in the membrane. It is possible that the reduction in PIP_2 by PGE_2 can then decrease the activity of ECaC1 directly or indirectly by enhancing the sensitivity of ECaC1 to inhibition by acid.

Sodium Load. As described above, the reabsorption of calcium in the proximal tubule and TAL is passive and coupled to sodium reabsorption (30). Sodium load and volume expansion inhibit reabsorption of sodium and calcium in these nephron segments and increase renal calcium excretion.

Clinical Conditions Associated with Renal Hypercalciuria

High Animal Protein Diet. A high animal protein diet has long been known to cause hypercalciuria. Several mechanisms have been invoked. It may partly be due to enhanced bone loss (see Resorptive Hypercalciuria). Hypercalciuria of dietary acid excess does not appear to be intestinal in origin since there is no change in intestinal calcium absorption (31). High dietary protein intake causes glomerular hyperfiltration, which causes increased filtered load of calcium. The hypercalciuria, however, is above and beyond that of an increased filtered load to the kidney, indicating that a high dietary protein intake also causes a direct inhibition of calcium reabsorption in the kidneys (32). The possibility that hypercalciuria of animal protein excess is due to an acid-mediated renal calcium leak (33) is supported by studies to be described below.
Dietary Acid Load. On a daily basis, the normal Western diet generates about 1 mEq/kg of acid in adult human beings. The kidney is responsible for clearing the systemic acid load. However, there is a gradual reduction in overall renal function with age, which reduces the ability of the kidneys to excrete acid (34). When combined with a continued intake of animal proteins (contain acid-generating components such as methionine or cystine), a slight but significant acidemia may persist in the elderly. Even in younger individuals, overindulgence of animal proteins, can produce a degree of systemic acidity. The ensuing metabolic acidosis or acid load can produce marked hypercalciuria.
In a recent clinical study, a high protein-low carbohydrate weight reducing diet increased net acid excretion by 54 mEq/day and reduced urinary pH by 0.5 unit (35). While urinary calcium increased by 90 mg/day, intestinal calcium absorption was not altered and changes in bone markers were unremarkable. In another study, an animal model of animal protein excess was produced in rats by feeding a high casein diet (32). Compared with a low casein diet, urinary calcium was 3-4 fold greater on a high casein diet that was high in acid ash content. In a preliminary study (unpublished observations, Preisig et al.), the neutralization of the acid load by co-administration of potassium citrate completely abrogated the rise in urinary calcium from the high casein diet.

PGE_2 Excess. It is known that high dietary protein intake increases renal production of PGE_2. In experimental animals, in-

tra-arterial administration of PGE_2 increased urinary calcium without changes in the systemic blood pressure or glomerular filtration rate (36). Treatment with prostaglandin synthetase inhibitors restored normal urinary calcium among patients with hypercalciuria.
The complete abrogation of hypercalciuria of high animal protein diet by administration of alkali mentioned earlier suggests that an increase in acid load is the principal factor for hypercalciuria of high dietary protein intake. PGE_2 may exacerbate hypercalciuria by increasing the sensitivity of ECaC1 to inhibition by acid.

Dietary Sodium Load. A high dietary intake of sodium is well known to increase urinary calcium by impairing renal tubular reabsorption of calcium (37). An increment in dietary sodium of 100 mEq/day increases urinary calcium by about 40 mg/day. While the slope is not altered, the intercept is higher in patients with nephrolithiasis for each increment of sodium intake; thus, there is higher degree of hypercalciuria (38). The induced hypercalciuria normally produces a compensatory rise in intestinal calcium absorption, probably by stimulating parathyroid function and the synthesis of 1,25(OH)2D3.

Relative Hypoparathyroidism from Enhanced Intestinal Calcium Absorption. In hypoparathyroidism, correction of hypocalcemia by vitamin D often produces hypercalciuria.

Estrogen Deficiency of Postmenopausal State. Several clinical studies suggest that urinary calcium is increased in the postmenopausal state, possibly due to estrogen lack. Among women with stones, the urinary calcium significantly increased during the 6th decade of life, coinciding with a rise in stone formation rate (39). Compared with the untreated postmenopausal women, those treated with estrogen had reduced urinary calcium (fasting and 24-hour). The rise in urinary calcium following menopause appears to be renal in origin (40). Sakhaee et al. reported on a group of postmenopausal women with osteoporosis who displayed biochemical picture of renal hypercalciuria with secondary hyperparathyroidism, associated with high bone resorption on bone histomorphometric analysis (40). Nordin et al. found that urinary calcium (corrected for creatinine) was significantly greater in normal postmenopausal women who had not received hormone replacement therapy, compared with normal premenopausal women (41). The higher urinary calcium in postmenopausal women could not be explained by increased filtered load of calcium. Lastly, McKane et al. directly measured serum ultrafilterable calcium and tubular reabsorption of calcium in early postmenopausal women before and following 6 months of estrogen therapy (42). After the effect of changes in endogenous PTH secretion was excluded by a pharmacologic dose of PTH, estrogen treatment significantly increased the tubular reabsorption of calcium, providing evidence that estrogen may directly lower urinary calcium independently of PTH. Thus, the available clinical data suggest that renal calcium leak is present in estrogen deficiency, and is corrected by estrogen therapy.

Mutations of ECaC Genes as a Potential Cause of Renal Hypercalciuria. As described above, ECaC1 and ECaC2 are critical gate-keepers of transcellular Ca^{2+} transport and primary targets for regulation of renal calcium handling by calcitropic hormones, dietary factors, and acid-base status. It is thus tantalizing to speculate that mutations of these genes may be responsible for genetic forms of renal hypercalciuria. In nine families with hypercalciuric nephrolithiasis, no mutations were identified in the exons of *ECaC.* Haplotype analysis did not implicate a role of the locus on chromosome 1. Single nucleotide polymorphisms were noted in the 5'-flanking region. No genotype-phenotype association was identified (43). Since there is likely loci heterogeneity, ECaC is not entirely ruled out as a candidate gene for renal hypercalciuria.

Pathophysiology of resorptive hypercalciuria (Table III)

Table III - Pathophysiology of Resorptive Hypercalciuria.

1. Primary Hyperparathyroidism
2. Base Changes in AHRAC as a Part of AH Syndrom
3. Dietary Acid Load

In resorptive hypercalciuria, the primary defect responsible for hypercalciuria is believed to be excessive bone resorption. The prototype of resorptive hypercalciuria is primary hyperparathyroidism (PHPT) but it may also be seen in association with AH and dietary animal protein excess.

Primary Hyperparathyroidism

In PHPT, a hypersecretion of PTH from a benign solitary parathyroid adenoma (80%) or multiglandular parathyroid hyperplasia (15%) produces excessive bone resorption. Under normal circumstances, an increase in circulating ionized calcium is followed by a rapid decrease in PTH secretion. In PHPT with adenoma, this feedback control is impaired, resulting in hypersecretion of PTH. In PHPT caused by hyperplasia, the sensitivity to circulating calcium is relatively intact but the number of parathyroid cells is increased, enhancing PTH secretion. In either case, the PTH excess increases the number of active osteoclasts on the bone surface, stimulating bone resorption. The resulting rise in serum calcium increases the renal filtered load of calcium, causing hypercalciuria. Although PTH augments renal tubular reabsorption of calcium, hypercalciuria ensues from the greatly increased filtered load of calcium and from a suppressive effect of hypercalcemia on calcium reabsorption (44).
PTH also induces the renal 25-hydroxyvitamin D_3-1α-hydroxylase; thus, serum $1,25(OH)_2D_3$ concentration is often high in PHPT. The enhanced $1,25(OH)_2D_3$ synthesis stimulates osteoclastic bone resorption and, more importantly, raises intestinal calcium absorption. These effects further increase the circulating concentration of calcium and contribute to hypercalciuria. Thus, hypercalciuria of PHPT is primarily resorptive and secondarily absorptive in origin.

Base Changes in AHRAC as a Part of AH Syndrome

Despite intestinal hyperabsorption of calcium, patients with AH tend to have negative calcium balance (45) and low spinal bone mineral density (6). The most notable reduction in spinal bone density was seen in patients with fasting hypercalciuria, implying a role for resorptive hypercalciuria (46). The association between dietary risk factors (such as high sodium and acid intake) and lower bone density is attenuated in AH patients with the most severe fasting hypercalciuria, suggesting presence of intrinsic bone defects in this subgroup of AH patients (6). There is a 4-fold higher risk of fractures in patients with hypercalciuria (47). Bone biopsies in patients with AH have shown a picture compatible with low bone formation and turnover; less common are features suggestive of increased bone resorption (48). In concert, these findings

provide compelling circumstantial evidence for bone involvement in AH.
At the molecular level, the number of base changes in AHRAC has been shown to be well correlated well with reduced spinal bone density in AH (14). Among AH patients with intestinal hyperabsorption of calcium, patients harboring *AHRAC* base changes had much lower bone density than those with wild type *AHRAC* genotypes (14). While *AHRAC* is expressed in bone, its current function in osteoblasts and osteoclasts are unknown. It is conceivable that dysfunction of *AHRAC* can alter the rate of bone formation that produces an inappropriately high bone resorption in AH.

Dietary Acid Load

Besides impairing renal tubular reabsorption of calcium, metabolic acidosis causes bone loss by physicochemical and cellular effects. When calvaria devoid of live bone cells are exposed to an acid medium, calcium is released from dissolution of bone mineral (calcium phosphate) (49). Calcium mobilization from live calvaria is more marked due to stimulation of osteoclastic bone resorption and inhibition of osteoblastic bone formation (50).
In the previously cited animal model of animal protein excess (32), we also evaluated the effect of chronic metabolic acidosis on bone remodeling. On bone histomorphometric analysis, a high casein diet with abundant acid ash content significantly increased bone resorption and turnover (eroded surface, osteoclastic surface, mineralizing surface, and double layered tetracycline surface), compared with a low casein diet (unpublished observations, Zerwekh, Preisig et al.). Bone formation (osteoblast surface) was unaffected. Thus, there was a net bone loss indicated by low bone volume, and reduced trabecular and cortical thickness. In a preliminary study, the addition of potassium alkali to the high casein diet returned above changes toward the values of the low casein diet.
Thus, dietary acid load produces high bone turnover and bone loss, contributing to hypercalciuria.

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